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A RABBIT MODEL OF INHALATION INJURY

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In the course of developing a model of inhalation injury, the relationship between the severity of pulmonary injury and specific techniques and doses of smoke exposure was examined in pairs of rabbits simultaneously exposed to smoke. In group I (5 pairs), one animal in each pair was exposed to smoke with a breath hold (BH) at the end of each exposure; the second animal received an exposure producing the same level of carboxyhemoglobin without BH. In group II (6 pairs), both animals were exposed to 25 units of smoke simultaneously, with BH. In group III (3 pairs), one animal received a 20-unit exposure and the other a 25-unit exposure, both with BH. In group IV, 9 animals received 25-unit exposures with BH and were observed for 4 days. Groups V and VI served as controls. Smoke exposure with BH regularly produced severe injury in terms of decreased Pa_{O_2} and histopathologic changes, while exposure without BH did not, despite high levels of carboxyhemoglobin after smoke inhalation. The mean differences in percent residual Pa_{O_2} (Pa_{O_2} at 48 hours × 100/pre-injury Pa_{O_2}) and in extravascular lung water (EVLW) at 48 hours within pairs of animals receiving 25 units with BH were 12.3% ± 5.33%, and 0.271 ± 0.157 mL/g, respectively. Histologic findings such as necrotic tracheobronchitis with pseudomembrane were consistently present. No differences were observed between animals receiving exposures of 20 and 25 units. During the 4 days of observation, three animals in group IV died. Pa_{O_2} was lowest on the second day and rose thereafter in all surviving animals except in one that had massive pneumonia. Extravascular lung water levels were still elevated on the fourth day after injury. Histologically, the destroyed surface epithelium in the airway was covered by a nonciliated epithelium, and focal pneumonia was found frequently in the pulmonary parenchyma. These results indicate an advantage of the extended exposure afforded by BH in creating consistent, severe injury and the important part played by pneumonia in determining prognosis beyond the second postinjury day. The model appears useful for evaluating the effects of inhalation injury with concurrent cutaneous burn or wound infection, and for assessing various regimens for the treatment of inhalation injury.

IN RECENT YEARS, great advances in clinical management and the prevention of burn wound infection have enhanced the survival of severely burned patients. The prognosis of large cutaneous burns is still compromised, however, when complicated by concurrent smoke inhalation injury. Inhalation injury, especially when combined with pneumonia, remains a major contributor to the morbidity and mortality of burn injury.[1]

Several animal models[2-6] have been developed to study the pathophysiology of inhalation injury. We selected the rabbit as the experimental animal for this model for several reasons. First, the species lends itself to studies of the interaction between inhalation injury and cutaneous burn, since a rabbit's size facilitates the use of immersion in hot water to produce burn injuries of precise size and depth. Second, the animal is large enough to permit successive blood samples, which are necessary for the analysis of the time course of physiologic response. Finally, the animals are easy to maintain and handle, and their cost is not high.

Inhalation injuries are usually produced by gaseous or particulate products of incomplete combustion. Particles in smoke may be coated with irritating chemical agents, such as aldehydes and hydrogen chloride, and thus carry these irritants as far as the alveoli.[7] Incorporation of a 0.5-μm pore-size filter significantly blunts the changes in lung mechanics observed after smoke exposure.[8] Generation of such toxic elements depends on conditions of combustion such as oxygen supply, temperature, and heating rate in the fire.[9] Since each fire generates its own variety of smoke and associated toxic materials, the severity of experimental inhalation injury is not completely controllable.

In pilot studies, we found that administration of smoke to rabbits in the usual manner did not produce consistent injury, although carboxyhemoglobin levels in arterial blood taken immediately after smoke exposure were be-

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tween 60% and 75%. A spectrum of severity of injury was observed at constant doses of smoke generated from the same material.

For these reasons, we studied the effects of a breath hold at the end of each smoke inhalation on the consistency of severity of injury. To assess the variance inherent in this model, we exposed pairs of rabbits to the same or different doses of smoke simultaneously. Finally, we studied the longer term effects of smoke exposure in these animals, assessing mortality, morbidity, and histopathologic changes.

MATERIALS AND METHODS

Animals

Forty-seven male New Zealand white rabbits (mean weight 3050 ± 380 g) were used in this study. The animals were housed singly in stainless steel cages and were studied in an unanesthetized state for 2 or 4 days with food and water provided ad libitum. Intravenous fluid was not administered during the experiments.

Six groups of rabbits were studied. In group I (5 pairs), pairs of animals were simultaneously exposed to smoke. One received 25 units of smoke with a breath hold (BHU) at the end of each smoke insufflation. One unit of smoke required 27 seconds to administer and consisted of three successive insufflations of smoke (1 second) with a breath hold of 4 seconds, followed by 12 successive ventilations with air (Fig. 1A). The other member of the pair received 8 units of smoke without breath hold, each unit consisting of 15 successive insufflations with smoke, followed by 12 ventilations with air (Fig. 1B). Tidal volume was fixed at 12 mL/kg for all ventilations. In group II (6 pairs), members of a pair of animals were simultaneously exposed to 25 BHU and observed for 2 days. In group III (3 pairs), one animal in each pair was exposed to 20 BHU and the other to 25 BHU. In group IV (n = 9), animals were individually exposed to 25 BHU and pulmonary changes were observed for 4 days. Groups V (n = 5) and VI (n = 5) served as sham-operated controls and were observed for 2 and 4 days, respectively.

Methods

A 24-gauge catheter was placed in the middle artery of the ear a day before an experiment. Animals were anesthetized with ketamine (40–45 mg/kg) administered intramuscularly, followed by an additional dose of pentobarbital (10–20 mg/kg), and intubated. Muscle relaxant (pancuronium bromide, 0.01 mg/kg) was administered before smoke exposure. Blood gas levels were determined (BGElectrolytes, Instrumentation Laboratory, Lexington, Mass) before the experiment and every 24 hours after injury. Carboxyhemoglobin concentrations (CO-Hb) were measured in arterial blood taken immediately after smoke exposure, using a Co-Oximeter (Model 282, Instrumentation Laboratory). After euthanasia, the right lung was removed rapidly for measurement of extravascular lung water (EVLW) by a modification of the gravimetric method described by Pearce et al.[10] Lung homogenate was dried in a microwave oven (Model MDS-81D, CEM Corp., Matthews, NC) by the method of Peterson et al.[11] The left lung was fixed for histologic examination.

(A) One unit of smoke with breath hold (BHU)

Smoke (15 seconds) 1, hold , 2, hold , 3, hold .

Air (12 seconds) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.

(B) One unit of smoke without breath hold

Smoke (15 seconds) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15.

Air (12 seconds) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12.

Figure 1. The "unit" of smoke.

Intubation Technique

For accurate and reliable endotracheal intubation, a string was used to guide a tube from the mouth to the trachea. A 1-inch midline skin incision was made on the ventral surface of the neck and the trachea was exposed. The glottis was anesthetized, using 0.1 mL 4% Xylocaine injected through an 18-gauge needle inserted into the trachea 1 cm below the thyroid cartilage. A guide wire with 4-0 suture was passed cephalad from the needle hole in the trachea into the mouth. This suture was tied to a suture attached to the tip of a 3-0 cuffed endotracheal tube. The tube was then drawn caudad by traction on the lower suture until correct placement in the trachea was reached. If resistance was encountered at the larynx, the tube was rotated gently, or the position of the head was changed to allow the bevel of the tube to pass between the vocal folds. After confirmation of hemostasis, the incision was closed.

Smoke Inhalation

Smoke was produced in a generator by burning seven and a half commercially available disposable pads (Stanford Professional Products Corp., Pennsauken, NJ) made of cellulose (83.4% by weight), polyethylene (8.8%), polypropylene, etc. Smoke was collected in a Douglas Bag (50 L, Harvard) through a smoke delivery system and cooled to room temperature. Carbon monoxide (CO) concentration was measured in the collected smoke (CO 101, Neotronics, Gainesville, GA), and if the level was outside a range of 1% to 2%, the process was repeated until smoke having the desired concentration was obtained. A volume-adjustable syringe was used for alternate insufflation of smoke or air.

Statistical Analysis

The data are presented as means ± standard deviation. Statistical differences in the change of Pao_2 within and between groups were evaluated using the Tukey studentized range method, after a two-factor ANOVA with repeated measures on one factor. The differences in EVLW on the second and fourth days postinjury were evaluated using the Tukey studentized range method after one-factor ANOVA. Null hypotheses were rejected at $p < 0.05$.

RESULTS

Group I

One animal died 26 hours after 25 units of smoke exposure with breath hold and this pair was excluded from analysis. The average immediate postexposure CO-Hb concentrations in rabbits receiving 8 units without BH and 25 units with BH were 68.1% ± 2.58% (SE) and 69.0% ± 4.10%, respectively. Changes

of Pa_{O_2} in each group are shown in Figure 2. Only rabbits exposed to smoke with breath hold had significantly lower Pa_{O_2} than the control at 24 or 48 hours. Extravascular lung water levels increased in rabbits exposed to smoke with or without breath hold; there was no statistical difference between the two (Fig. 3). Smoke exposure with BH consistently produced necrotic tracheobronchitis and bronchiolitis with pseudomembrane formation. Inflammation of surrounding tissue, including edema and increased accumulation of neutrophils, was also present. Histologic changes were more severe in the trachea and major bronchi than in the distal bronchioles or parenchyma. Most of the rabbits exposed to smoke without BH had only minimal focal damage.

Group II

The average of CO-Hb levels after smoke exposure in all rabbits was 75.4% ± 2.98%. The mean difference of CO-Hb levels within a pair was 2.67% ± 1.2%. The average Pa_{O_2} in all animals before and 24 and 48 hours after smoke exposure was 80.9 ± 6.67, 67.5 ± 8.56, and 57.0 ± 10.2 mm Hg, respectively. Figure 4 shows the paired differences in percent residual Pa_{O_2} (Pa_{O_2} × 100/preinjury Pa_{O_2}) at 24 and 48 hours after injury; the mean differences within a pair were 11.1% ± 10.1% and 12.3% ± 5.33%, respectively. The average of EVLW in all rabbits was 3.655 ± 0.229 mL/g; the mean difference of EVLW within a pair was 0.271 ± 0.157 mL/g.

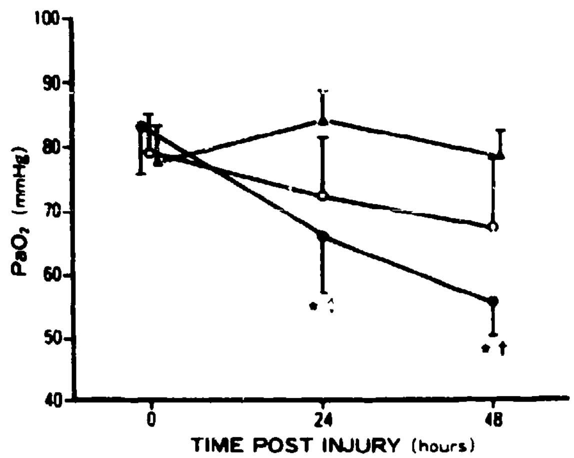

Figure 2. Time course of change in Pa_{O_2} for 48 hours after injury. Closed triangles: control; open circles: animals exposed without breath hold. Closed circles: animals exposed with breath hold. *Significantly different (p < 0.05 or more) from control values. †Significantly different from pre-injury values.

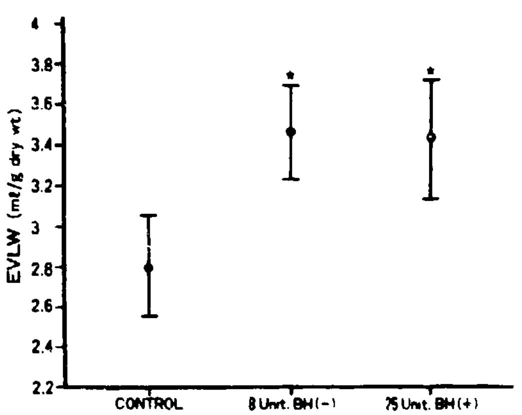

Figure 3. Extravascular lung water at 48 hours after injury. *Significantly different (p < 0.05 or more) from control values.

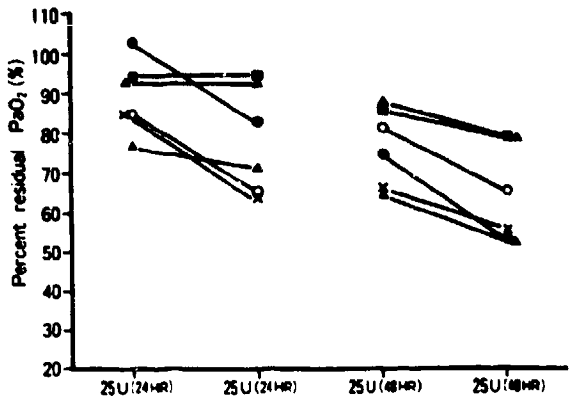

Figure 4. Percent residual Pa_{O_2} in paired animals at 24 and 48 hours after 25 BHU. Lines connect pairs.

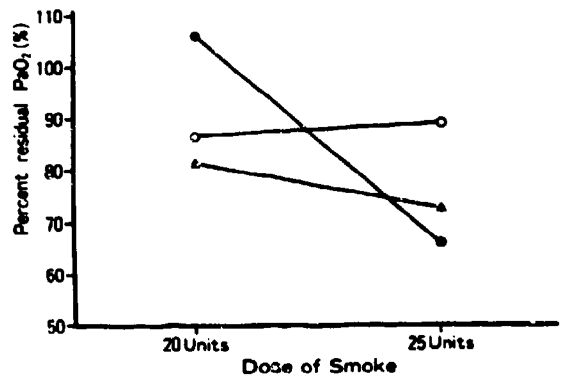

Figure 5. Percent residual Pa_{O_2} at 48 hours in paired animals exposed to 20 and 25 BHU. Lines connect pairs.

Group III

The percent residual Pa_{O_2} at 48 hours after injury in paired rabbits receiving 20 and 25 BHU is depicted in Figure 5. One rabbit did not experience any decrease of Pa_{O_2} and in one pair 20 units of smoke exposure produced more histologic evidence of damage than 25 units. No consistent difference in severity of injury was identified in this group.

Group IV

The average of CO-Hb levels in all rabbits immediately after smoke exposure was 72.5% ± 6.83%. Three of nine animals died during the 4-day period of observation (Fig. 6). In those animals that died, wheezing was always present, and death was sudden. In all the surviving animals, Pa_{O_2} decreased gradually and was lowest on the second day, rising thereafter except in one rabbit with severe bilateral pneumonia (Fig. 7). The EVLW level at the fourth day was 3.533 ± 0.371 mL/g and was still high in the animals exposed to smoke compared with EVLW of the control animals (Fig. 8). Histologically, the injured tracheobronchial surface was covered by a nonciliated, stratified epithelium, with some areas still covered with pseudomembrane. The extent of subepithelial edema and the numbers of infiltrating inflammatory cells on day 4 was markedly less than on the second postinjury day. In contrast to the reparative

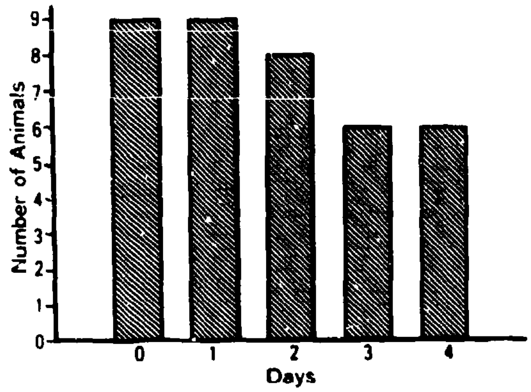

Figure 6. Survival during 4 days after 25 units of smoke exposure.

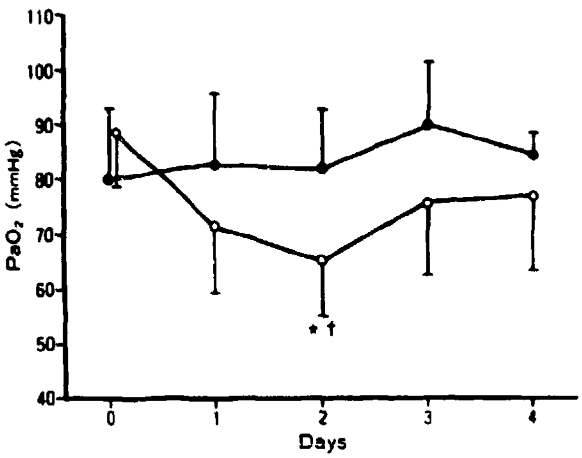

Figure 7. Time course of change in PaO_2 for 4 days after injury. Closed circles: control; open circles: animals exposed to 25 BHU. *Significantly different ($p < 0.05$) from control values. †Significantly different ($p < 0.05$) from pre-injury values.

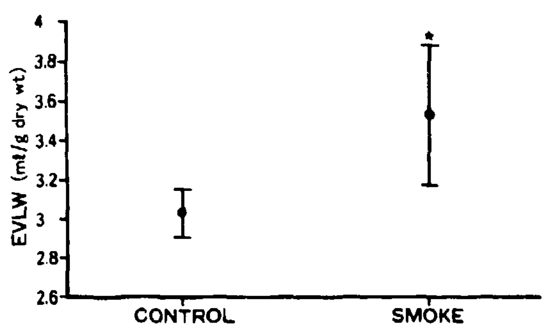

Figure 8. Extravascular lung water at 4 days after injury. *Significantly different ($p < 0.05$) from control values.

processes in the trachea and bronchi, scattered focal areas of pneumonia were frequently found in the parenchyma.

DISCUSSION

In human and experimental studies of inhalation injury,[12,13] histologic changes in the lung characteristically include loss of cilia and respiratory epithelium. Sloughing necrotic tissue and inflammatory exudate lead to pseudomembrane formation and airway obstruction with resultant atelectasis, congestion, and edema. Coincident with these changes, progressive hypoxia develops and the risk of life-threatening bacterial infection is increased.[14] Rabbits exposed to smoke in this study showed similar changes.

The use of rabbits in inhalation studies is not widespread, partly because of difficulty in maintaining a patent airway and adequate levels of anesthesia. The susceptibility of rabbits to anesthesia varies widely and the margin between surgical anesthesia and death is quite narrow.[15] Pentobarbital is the most commonly used intravenous anesthetic agent for rabbits, and has the serious disadvantage of depressing respiration; this is the principal cause of death.[16] The small glottis and larynx hidden behind the tongue are major obstacles to rapid intubation. The intubation technique used in this study was simple to perform without injury to the glottis and required minimal anesthetic doses. No complications attributable to anesthesia were encountered.

Potkin et al.[3] observed severe hypoxemia and pathologic changes in rabbits after 40 minutes exposure to white pine wood smoke diluted by air, with a resultant mean CO-Hb level of 39.6%. Long-term exposure to smoke with low CO concentration will permit severe injury without high CO-Hb levels. We used smoke with relatively high concentrations of CO to render exposure time as short as possible because, in a pilot study, smoke with high CO levels produced more severe injury than smoke with low CO concentration, even when the same dose was administered. Severity of injury did not appear to be better controlled by long-term exposure, because of the technical difficulty in generating and administering smoke of constant quantity and quality.

Breath hold has been used in only one previous study of inhalation injury[4] and its effect has not been well defined. In the present study, severe pulmonary damage was not produced consistently without breath hold, and even with similar levels of CO-Hb, exposure to smoke with breath hold produced more severe injury in terms of oxygenation and histologic changes. The total number of smoke insufflations was smaller in rabbits receiving 25 units with a breath hold than in those exposed to 8 units without a breath hold (75 vs. 120), while exposure time was longer in the former animals (375 vs. 120 seconds). This result suggests that almost all CO in smoke may be absorbed during the early phase of a breath hold, while other combustion products may continue to be deposited in the pulmonary tree during the later phase of the hold, resulting in increased absorption of other combustion products and greater toxicity to the exposed tissue.

The factors necessitating this manner of smoke exposure, which may differ from those affecting patients, are not known. The material burned in this study was mostly cellulose, the principal decomposition products of which are acrolein and other aldehydes.[17] These products are present in high concentration in smoke from the com-

bustion of household materials and are known to cause acute pulmonary injury.[18] Although we did not measure combustion products from the pads, it is unlikely that smoke from this material is in any sense uniquely benign or unlikely to produce severe injury. Differences in the velocity of CO-Hb saturation resulting from differences in blood volume, cardiac output, or affinity of rabbit hemoglobin for CO, or differences in sensitivity of the respiratory system to combustion products may offer possible explanations. It is possible that patients exposed to smoke with high CO levels do sustain only slight injury if they survive and that the observed clinical correlation between CO-Hb levels and severity of inhalation injury pertains only to patients exposed to smoke having relatively low CO concentrations.

Despite the considerable differences observed in Pa_{O_2} and histology, EVLW was equally elevated in animals exposed to smoke with and without breath hold, suggesting that the causes of these indices of injury may differ. In a sheep model of inhalation injury, the increase of lung lymph flow has been reported to correlate well with CO-Hb level after smoke exposure, while diminution in the ratio of the Pa_{O_2} to the fraction of inspired O_2 (Pa_{O_2}/F_{IO_2}) does not.[19] It has been reported that rabbits exposed to carbon monoxide, with a resultant CO-Hb level of 63%, show widespread areas of both epithelial and endothelial swelling and marked interstitial edema without an increase in the alveolar/arterial O_2 difference.[20] Our results are consistent with these findings and suggest that carbon monoxide in smoke may be related to edema formation.

In a pair of animals receiving the same dose of smoke simultaneously, small differences of CO-Hb were detected and were probably attributable to individual differences in cardiac output, blood volume, etc. The average differences in residual percent Pa_{O_2} and EVLW at 48 hours postinjury were 12.3% ± 5.33% and 0.271 ± 0.157 mL/g, respectively. These differences are probably related to individual biologic variation, since heterogeneity in the smoke itself was excluded by pairing, and other problems, such as technique and apparatus, are regarded as negligible because of small differences in CO-Hb levels within a pair. Beeley et al.[21] recognized marked variability in the extent of histologic changes in the lungs of rabbits despite uniform exposure to acrolein in terms of time and concentration. Even with the addition of a uniform inhaled volume we still found modest differences in Pa_{O_2} and EVLW within pairs. In a sheep model of inhalation injury,[4] the curve of physiologic response with increasing dose is sigmoid in shape; beyond a certain dose of smoke, deterioration of pulmonary function accelerates rapidly toward lethality. The difficulty in precise control of severity may be a consequence of this characteristic of the physiologic response.

Animals died between 24 and 72 hours; no deaths occurred before or after this period. It appeared that the immediate cause of death was not hypoxia owing to impairment of alveolar structure, but partial obstruction of the trachea or major bronchi. Wheezing was recognized in all animals before death and the animals' condition deteriorated suddenly just before death. Massive sloughing casts in the trachea may have separated and occluded a lower airway already narrowed by pseudomembrane formation. Although we could not demonstrate complete obstruction of the trachea, compromised animals with little pulmonary reserve may die of acute partial obstruction. The Pa_{O_2} fell progressively and was lowest on the second day; it rose thereafter in all animals except one that had extensive pneumonia. Pathologic changes in trachea, bronchi, and bronchioles also improved by the fourth postinjury day. Peitzman et al.[22] have shown lung water accumulation in patients with inhalation injury, whereas Tranbaugh et al.[23] could detect edema only after the patients became septic. Recent studies support an increase of lung water during the acute phase of injury.[24,25] Our results confirm an increase in lung water 2 days after injury, with edema observed on the fourth day. This finding is consistent with human studies in which patients recovered during the first 3 days except for elevated EVLW.[25]

In the experimental model of inhalation injury described here, the differences in Pa_{O_2} and EVLW within a pair were small and, as long as massive pneumonia did not occur, the time course of Pa_{O_2} showed consistent improvement beyond the second day. This model appears useful for the evaluation of the effects of various treatments on inhalation injury, and for the assessment of the consequences of interactions among burns, infection, and inhalation injury.

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